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Method for Trace Level Analysis of C8, C9, C10, C11, and C13 Perfluorocarbon Carboxylic Acids in Water

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A method was developed for the trace level analysis of pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), heneicosafluoroundecanoic acid (C11) and pentacosafluorotridecanoic acid (C13) in water. Samples were concentrated by solid-phase extraction (SPE) before analysis by combined liquid chromatography/electrospray tandem mass spectrometry (LC/MS/MS). A surrogate standard, 9-hydrohexadecafluorononanoic acid (9H), was used to monitor recovery. The lower limit of quantitation (LLOQ) for C8, C9, C10, C11, and C13 was determined to be 25 ng/L in water. The specificity of the method was established by showing no significant interferences (<20% of the LLOQ standard) in control samples of well, stream, spring, tap, omnisolve, and type I water at the retention time of the target analytes. The linearity of the method was determined; the coefficients of determination for the five calibration curves generated were all >0.985. Good within-day and between-day accuracy and precision were demonstrated. Extracts and standards were shown to be stable after remaining at room temperature for ~24 h. Samples fortified with C8, C9, C10, and C11 were shown to be stable after remaining at room temperature for 14 days before extraction. Samples fortified with C13 were shown to be stable after remaining at room temperature for 7 days before extraction. Fortified samples, extracts, and standards demonstrated stability after being stored in a refrigerator for 14 days for all analytes. Long-term storage stability was demonstrated for methanolic stock solutions.

Fluorinated alkyl surfactants have a wide variety of uses in various industrial products, including plastics, surface treatments, lubricants, and fire-fighting foams.[1−4] These materials are usually partially or completely fluorinated sulfonic or carboxylic acids or their salts. Of the completely fluorinated materials, perfluorinated carboxylates are used in the manufacture of such fluoropolymers as poly(tetrafluoroethylene) (PTFE), fluorinated ethylene−propylene (FEP), polyvinylidene fluoride (PVDF), and fluoroelastomers, and a combination of perfluorinated sulfonates and carboxylates are used in fire-fighting foams. The carboxylates used in fluoropolymer manufacture are also sometimes referred to as fluoropolymer polymerization aids (FPAs).[2]

Both perfluorinated alkyl sulfonates and carboxylates are extremely stable and resistant to degradation,[1] which has led to concern about persistence in the environment and in biological matrixes. There is a need for trace level analysis of the perfluorinated sulfonates and carboxylates in the various media. Prior analytical work on sample handling and methodology has been reviewed elsewhere.[1−4]

Several recent studies have reported on trace level analysis of FPAs in groundwater as a result of spills of fire-fighting foams,[5,6] surface water in relation to a plant manufacturing fluoropolymers,[7] and as a result of an accidental release of fire-fighting foam.[8,9] In all cases, the analytical method used was liquid chromatography coupled with tandem mass spectrometry, LC/MS/MS. This is a desirable method for trace level analysis, because it is specific to the compounds being analyzed, and it can achieve very low levels of quantitation.[2] The analytes in the studies cited above were perfluorohexanoic acid (PFHA, or C6), perfluorooctanoic acid (PFOA, or C8), perfluorooctansulfonate (PFOS), and in one case,[8] perfluorododecanoic acid (PFDoA, or C12).

This paper describes a method and its validation for the determination of pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), heneicosafluoroundecanoic acid (C11), and pentacosafluorotride-

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(1) Kissa, E. *Fluorinated Surfactants and Repellents*, 2nd ed.; Marcel Dekker: New York, 2001.

(2) *Detecting and Quantifying Low Levels of Fluoropolymer Polymerization Aids—A Guidance Document*; The Society of the Plastics Industry, Inc.: Washington, DC, 2003.

(3) Schultz, M. M.; Barofsky, D. F.; Field, J. A. *Environ. Eng. Sci.* **2003**, *20*, 487.

(4) Schröder, H. F. *J. Chromatogr., A* **2003**, *1020*, 131.

(5) Moody, C. A.; Field, J. A. *Environ. Sci. Technol.* **1999**, *33*, 2800.

(6) Moody, C. A.; Hebert, G. N.; Strauss, S. H.; Field, J. A. *J. Environ. Monit.* **2003**, *5*, 341.

(7) Hanson, K. J.; Johnson, H. O.; Eldridge, J. S.; Butenhoff, J. L.; Dick, L. A. *Environ. Sci. Technol.* **2002**, *36*, 1681.

(8) Moody, C. A.; Kwan, W. C.; Martin, J. W.; Muir, D. C. G.; Mabury, S. A. *Anal. Chem.* **2001**, *73*, 2200.

(9) Moody, C. A.; Martin, J. W.; Kwan, W. C.; Muir, D. C. G.; Mabury, S. A. *Environ. Sci. Technol.* **2002**, *36*, 545.

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canoic acid (C13) in water. The method uses external standards for quantification by LC/MS/MS analysis, with a lower limit of quantitation of 25 ng/L, and it uses a surrogate standard, 9-hydrohexadecafluorononanoic acid (9H), to monitor recovery. Samples are concentrated by a factor of 8 during the analysis.

MATERIALS AND METHOD

Chemicals. Pentadecafluorooctanoic acid (C8, 99.9%), heptadecafluorononanoic acid (C9, 98.4%), nonadecafluorodecanoic acid (C10, 99.9%), and heneicosafluoroundecanoic acid (C11, 93.8%) were purchased from Sigma-Aldrich (Milwaukee, WI). Pentacosafluorotridecanoic acid (C13, 98.8%) was custom-synthesized by Sigma-Aldrich. 9-Hydrohexadecafluorononanoic acid (9H, 95%) was purchased from Daikin Fine Chemicals (Osaka, Japan). Reagent grade ammonium acetate and sodium thiosulfate were used. HPLC grade methanol was used, and methanol lots were checked for contaminants by LC/MS/MS before use; some lots were found unsuitable for use. Type I water, with a minimum electrical resistivity of 16.67 MΩ cm at 25 °C, was produced in-house from a Labconco Waterpro workstation. Type I water used for solution preparation and extractions was filtered through a Hypercarb guard column (Keystone) using an HPLC pump. Before use, the guard column was washed with ~25 mL of HPLC grade acetonitrile, then ~25 mL of HPLC grade methanol, followed by ~25 mL of type I water using the HPLC pump at ~2 mL/min flow rate. The filtered type I water eluate was then collected. This water is referred to as "filtered type I water" hereafter. It is recommended that the column be washed, as described above, after filtering ~2 L of type I water. A flow rate of 2−3 mL/min is recommended.

Solutions and Standards. To avoid contamination, disposable lab ware (tubes, pipets, etc.) was used and is recommended for preparation of all solutions and all sample handling. Poly(tetrafluoroethylene) or PTFE-lined containers or equipment, including PTFE-lined HPLC vials for the HPLC auto sampler, must not be used, because perfluorinated carboxylic acid surfactants are usually used in the preparation of PTFE.

Ammonium acetate solution (50 mM) was prepared by weighing 3.86 g of ammonium acetate and dissolving in 1 L of filtered type I water. The 50 mM solution was diluted by a factor of 25 to make the 2 mM solution used subsequently for chromatographic mobile phase A. Methanol solution (40%) was made by measuring 400 mL of methanol and adjusting the volume to 1 L with filtered type I water. Sodium thiosulfate solution (250 mg/mL) was made by dissolving 25 g in 100 mL of filtered type I water.

Analytical standards that were prepared were used for three purposes: calibration standards, laboratory control spikes, and matrix spikes. Calibration standards were prepared in filtered type I water and were used to calibrate the response of the detector used in the analysis. Laboratory control spikes were prepared in filtered type I water at concentrations corresponding to the lower limit of quantitation (LLOQ) and 10 times the LLOQ and were used to determine analytical recovery. Matrix spikes were fortifications prepared by spiking samples collected in the field with known concentrations and were used to evaluate the effect of the sample matrix on analytical recovery.

Stock solutions of 100 μg/mL of C8, C9, C10, C11, C13, and 9H were prepared by weighing out 10 mg of analytical standard (corrected for purity) and diluting to 100 mL with methanol in a 100-mL volumetric flask. These stock solutions were placed in 125-mL low-density polyethylene bottles and stored in a refrigerator at 2−6 °C. Stability studies (described in the Method Validation Section) showed that the stock solutions of C8, C9, C10, C11, and 9H could be stored under these conditions for 6 months from the date of preparation. The C13 stock solution was stable for 3 months under these conditions.

Table 1. Standards for a Typical Calibration Set

concn, fortification soln (ng/mL)	fortification vol (μL)	vol, fortified control sample (mL)	final concn, calibration std (ng/L)
0	0	40	0
10	100	40	25
10	200	40	50
10	400	40	100
100	100	40	250
100	200	40	500
100	400	40	1000

A mixed fortification solution of 1.0 μg/mL of C8, C9, C10, C11, and C13 was prepared by adding 1.0 mL of each of the 100 μg/mL stock solutions to a 100-mL volumetric flask and bringing up to the mark with methanol. Successive dilutions with methanol were made to prepare mixed fortification solutions of 0.1 μg/mL and 0.01 μg/mL concentrations.

Fortification standards of 9H at concentrations of 1.0 and 0.1 μg/mL were prepared by successive dilutions with methanol of the 100-μg/mL 9H stock solution. All fortification standard solutions were stored in a refrigerator (in 125-mL low-density polyethylene bottles) at 2−6 °C for a maximum of 3 months from the date of preparation, after which it is necessary to prepare new standards.

Calibration Standards. LC/MS/MS calibration standards were prepared in filtered type I water. The calibration standards were processed through the same solid-phase extraction procedure used for the samples. It is recommended that a zero standard solution (reagent blank) be prepared with each set of standards extracted. This standard is used to assess the reagents used to prepare the standards and is included as part of the calibration curve. It is recommended that the extracted standards be used for a period of no more than 2 weeks when stored refrigerated at a temperature between 2 and 6 °C. Table 1 is an example of standards prepared for a typical calibration set; additional concentrations may be prepared as needed.

Method. The flow diagram of the method is given in Scheme 1, followed by a detailed description of each step.

Sample Processing. Minimal sample processing was needed for water samples. It is recommended that samples be stored refrigerated between 2 and 6 °C and analyzed within 2 weeks of sampling. Samples stored refrigerated should be allowed to equilibrate to room temperature before analysis. All samples must be thoroughly mixed and any visible solids removed by centrifugation at ~3000 rpm before being sampled for extraction.

Sample Preparation. It is recommended that each batch of samples extracted (typically 20 or less) include at least one reagent control (method blank using filtered type I water) and two reagent controls fortified at known concentrations to verify procedural recovery for the batch. At least one sample per batch should be extracted in duplicate. At least one sample extracted should also

Scheme 1. Method Flow Diagram

Measure 40 mL of sample

(Fortify samples when designated)

↓

Fortify all samples with 9H surrogate standard

↓

C18 Solid Phase Extraction

↓

Final Volume (5 mL)

↓

LC/MS/MS Analysis

be separately fortified at a known concentration and carried through the procedure to verify recovery. Additional matrix spikes may be performed as necessary.

Extraction Procedure. Into a 50-mL polypropylene centrifuge tube was measured 40 mL of sample, which was then fortified with analyte as needed. All samples were fortified with the 9H surrogate. Surrogate fortifications were made at 250 ng/L, but they can be prepared at alternate levels as long as the amount in the standards and samples are the same at the time of analysis. The lid was replaced, and the tubes were mixed well. For tap water samples, and any samples suspected of containing chlorine, 200 μL of a 250-mg/mL solution of sodium thiosulfate was added to the 40-mL sample and mixed well to deactivate the chlorine *before fortification*. A Sep Pak Vac 6 cm^3 (1 g) C_{18} solid-phase extraction (SPE) cartridge, purchased from Waters Corp (Milford, MA), was conditioned by passing 10 mL of methanol through it, followed by 5 mL of filtered type I water at a flow rate of ~2 drops/s. The column was not allowed to run dry at any time.

Standards. A 40-mL portion of a standard to be extracted was loaded onto a conditioned C18 cartridge at a flow rate of ~1 drop/s. The eluate was discarded. The cartridge was then washed with ~5 mL of 40% methanol in water. This eluate was also discarded. The column was finally eluted with ~5 mL of 100% methanol. A 5-mL portion of eluate was collected into a graduated 15-mL polypropylene centrifuge tube (VWR, Bristol, CT) to give a final volume of 5 mL.

Samples. The sample was loaded onto the conditioned cartridge at a flow rate of ~1 drop/s. The eluate was discarded. The cartridge was then washed with ~5 mL of 40% methanol in water, and the eluate discarded. The cartridge was then eluted with ~5 mL of 100% methanol. This eluate was collected into a graduated 15-mL polypropylene centrifuge tube to give a final volume of 5 mL.

The standard or the sample was then ready for analysis using electrospray LC/MS/MS. It should be noted that both standards and samples are concentrated by a factor of 8 during the extraction procedure: initial volume = 40 mL, final volume = 5 mL.

LC/MS/MS System and Operating Conditions (Electrospray). The mass spectrometer (MS) was a Micromass Quattro Ultima (Micromass Ltd, Manchester, U.K.) with a Micromass electrospray interface. A Harvard infusion pump (Harvard Instruments, Holliston, MA) was used for tuning. The computer was a COMPAQ Professional Workstation AP200. Software was Windows NT running MassLynx 3.3. The high performance liquid chromatograph (HPLC) was a Hewlett-Packard (HP, Agilent Technologies, Palo Alto, CA) series 1100, consisting of an HP quat pump, HP vacuum degasser, HP autosampler, and HP column oven. A 4 × 10-mm Hypercarb drop-in guard cartridge (Keystone, Part no. 844017–400) was attached on-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase or HPLC system.

Table 2. HPLC Gradient Conditions

time (min)	% A (2 mM ammonium acetate in type I water)	% B (methanol)	flow rate (mL/min)
0.0	60	40	0.3
0.4	60	40	0.3
1.0	10	90	0.3
7.0	10	90	0.3
7.5	0	100	0.3
9.0	0	100	0.4
9.5	60	40	0.4
13.5	60	40	0.4
14.0	60	40	0.3
15.0	60	40	0.3

Table 3. Ions Monitored and Their Approximate Retention Times

analyte	mode	transition monitored	retention time (min)
C8	negative	413 → 369	~5.5
C9	negative	463 → 419	~5.6
C10	negative	513 → 469	~5.7
C11	negative	563 → 519	~5.8
C13	negative	663 → 619	~6.1
9H	negative	445 → 381	~5.3

The HPLC column was a Genesis C_8 (Jones Chromatography, W. R. Grace & Co., Columbia, MD), 2.1 × 50 mm, 4 μm. Column temperature was 35 °C; injection volume was 15 μL. Mobile phase A was 2 mM ammonium acetate in type I water. Mobile phase B was methanol. Gradient conditions are given in Table 2. The ions monitored and their approximate retention times are shown in Table 3.

Example MS Operating Parameters. In Table 4 are presented example MS operating values. Actual values will most likely vary from instrument to instrument. These values may also change over time, even when analyzing samples on the same instrument. The values are changed to optimize for greatest sensitivity.

The MS was tuned for each analyte by infusing a ~0.2 μg/mL standard solution of analyte (at 10 μL/min, using an infusion pump) via a "T" into a stream of mobile phase containing 40% methanol and 60% 2 mM ammonium acetate at 0.2 mL/min flow rate. The analyte was initially tuned for the parent ion and then tuned for the product ion. Once the instrument was tuned, the optimized parameters were saved as a tune file. This tune file was then used during routine analysis.

Performance Criteria. It is recommended that a standard solution be run on the LC/MS/MS corresponding to the estimated LLOQ (25 ng/L) in matrix and a signal-to-noise ratio be obtained for the analyte transition of at least 5:1 compared to a reagent blank. If this criterion cannot be met, the instrument operating parameters should be changed and optimized (or the injection

Table 4. Example MS Operating Parameters

analyte	dwell (s)	collision energy (eV)	cone (V)
C8	0.2	10	15
C9	0.2	10	20
C10	0.2	10	10
C11	0.2	10	15
C13	0.2	17	20
9H	0.2	15	20

Source	Set
capillary	3.0 kV
hexapole 1	0 V
aperture 1	0 V
hexapole 2	0 V
source block temp	100 °C
desolvation temp	300 °C

Analyzer	Set
LM res 1	14.0 V
HM res 1	14.0 V
IEnergy 1	1 V
entrance	−2 V
exit	2 V
LM res 2	14.0 V
HM res 2	14.0 V
IEnergy 2	2.0 V
multiplier	700 V

Gas Flows	Set
cone gas	~130 L/h
desolvation	~750 L/h

Pressures	Read Back
gas cell	~0.003 mbar

volume increased, if appropriate). It is recommended that the instrument be checked with several injections of low-level calibration standard prior to sample analysis to ensure peak area precision, shape, retention time, and resolution. The peak area precision should have a relative standard deviation (RSD) value of ≤10% and the retention times should have an RSD value of ≤2.5%.

Calibration Curve. To gather data for the calibration curve, the same aliquot (between 10 and 25 μL) of each extracted standard processed in matrix was injected into the LC/MS/MS, starting from the lowest level standard to the highest level prepared, using six or more concentration levels. The final concentration of these calibration standards was equivalent to 8 times the concentration of the initial standard due to the concentration during the SPE procedure.

Linear standard curves were used for quantitation. They were generated for each analyte by linear regression using $1/x$ weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software. It was allowed that any calibration standard found to be a statistical outlier by using an appropriate outlier test (and documented in the raw data and referenced in the final report) could be considered for exclusion from the calibration curve. The total number of extracted calibration standards that may be excluded, however, could not exceed 20% of the total number of extracted standards injected. It is recommended that the correlation coefficient, R, for calibration curves generated be ≥0.992 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

Sample Analysis. It is recommended that the same aliquot (between 10 and 25 μL) of each standard, sample, recovery, control, etc. be injected into the LC/MS/MS system. Standards corresponding to at least six or more concentration levels, starting with the LLOQ level or below, should be included in an analytical set. An entire set of extracted calibration standards should be injected at the beginning of a set, followed by extracted calibration standards interspersed approximately every 5−10 samples (to account for a second set of extracted standards). Alternatively, an entire set of extracted calibration standards may be included at the beginning and end of a sample set. In either case, extracted calibration standards should be the first and last injection in a sample set. All calibration standards, except for statistical outliers, should be used in the calibration curve.

The concentration of each sample/fortification/control was determined from the standard curve on the basis of the peak area of each analyte. The standard responses bracketed the responses of the residue found in each sample set. If necessary, the samples were diluted with methanol to give a response within the standard curve range.

Fortification recoveries falling within 70−130% were considered acceptable. The analysis performed during the method development included fortifications at 25 and 2500 ng/L of each analyte in type I water. It is recommended that the total holding time between sample collection and analysis not exceed 14 days. Extracted samples must be stored refrigerated between 2 and 6 °C until analysis.

Samples for which no peaks were detected (i.e., signal-to-noise ratio is <3) at the corresponding analyte retention times were reported as ND (not detected). Samples for which peaks were detected at the corresponding analyte retention times but at less than the LLOQ were reported as NQ (not quantifiable).

Acceptance Criteria. It is recommended that the following criteria be met to ensure the presence of C8, C9, C10, C11, and C13 in the analytical sample:

The chromatogram must contain the parent and daughter ions shown in Table 3.

Method blanks must not contain any analyte at levels greater than the LLOQ. If a blank contains analyte at levels greater than the LLOQ, then a new blank sample must be obtained and the entire set must be re-extracted.

Recoveries of laboratory control spikes and matrix spikes must be between 70 and 130% of their known values. If a control spike falls outside of the acceptable limits, the entire set of samples must be re-extracted. Any matrix spike outside 70−130% should be re-extracted. If a matrix spike fails recovery, the sample and associated matrix spike should be re-extracted and reanalyzed for confirmation. If the recovery is still out of range, report both values and flag the data as having matrix effects present.

Recoveries of the 9H surrogate standard must be between 70 and 130%. Any sample in which the surrogate recovery does not fall within this range must be re-extracted. If a surrogate fails recovery, the sample should be re-extracted and reanalyzed for confirmation. If the recovery is still out of range, report both values and flag the data as having matrix effects present. Any standard

that does not meet this requirement cannot be included in the calibration curve.

Any calibration standard found to be a statistical outlier by using an appropriate outlier test (documented in the raw data and referenced in the final report) may be excluded from the calculation of the calibration curve. The total number of extracted calibration standards that can be excluded, however, must not exceed 20% of the total number of extracted standards injected.

The correlation coefficient, R, for the calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). All the standards injected in an analytical set should be used to construct the calibration curve, with the exceptions for outliers noted in section 5 above. If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

Retention times between standards and samples must not drift more than ±4% within an analytical run. If retention time drift exceeds this limit within an analytical run, then the set must be reanalyzed.

Calculations. The equation given below was used to calculate the amount of analyte found (in ng/L, based on peak area) from the calibration curve (linear regression parameters) generated by the MassLynx program,

$$\text{analyte found (ng/L)} = \frac{\text{(peak area} - \text{intercept)}}{\text{slope}} \times \text{DF}$$

where DF = factor by which the final volume was diluted, if necessary

For samples fortified with known amounts of analyte prior to extraction, the following equation was used to calculate percent recovery:

$$\text{recovery (\%)} = \frac{[\text{total analyte found (ng/L)} - \text{analyte found in control (ng/L)}]}{\text{analyte added (ng/L)}} \times 100$$

Note: Subtract analyte found in control (ng/L) from analyte found (ng/L), if applicable.

Method Validation. The method was validated generally according to U.S. Food and Drug Administration (US FDA) guidelines[10] and US FDA GLP 21 CFR 58. There were some differences from the guidelines. These include the use of external reference standards; no independent analyses of the reference standards were made, but certificates of analysis were used; the reference standards were not characterized under 21 CFR 58.105. The protocol for accuracy was mean values within ±20% of the theoretical values and for precision was coefficients of variation of ≤20%. For stability studies, the protocol for stability was results within 30% of theoretical. The low concentration used in the stability studies used was twice the LLOQ. The automated data collection systems were not fully compliant with 21 CFR 58.130-(e).

(10) *Guidance for Industry, Bioanalytical Method Validation*; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001.

RESULTS AND DISCUSSION

Specificity. Six water (matrix) blank samples were obtained from six different sources and were analyzed according to the method, along with a calibration standard at the LLOQ of 25 ng/L. There were no significant interferences (<20% of area of the LLOQ standard) at the retention time of any of the target analytes in control samples of well, stream, spring, tap, Omnisolve, and type I water; results were below the LLOQ. Good precision (<10% RPD) was observed between LLOQ standards for each compound analyzed prior to and after the source samples.

Linearity. The linearity of the method was established by analyzing calibration standards ranging from 0 to 2500 ng/L. Included were a matrix blank (matrix sample processed without surrogate standard), a zero sample (matrix sample processed with surrogate standard), and seven concentrations of standards. Each concentration was injected five times, and the five resulting curves from the five data sets were processed separately. For each of the five data sets of standards, the coefficients of determination for C8, C9, C10, C11, and C13 were greater than 0.985, and all of the recoveries deviated <15% from the nominal concentration at concentrations other than the LLOQ. All recoveries for the LLOQ level deviated <20% from the nominal concentration, except for one instance in one data set of a C10 recovery of 125% and one instance in one data set of a C11 recovery of 121%.

LLOQ. The lower limit of quantitation (LLOQ) of the method was determined by analyzing three replicates of a control sample and three replicates of fortified samples at 25 ng/L. The LLOQ was justified at 25 ng/L for water with fortification recoveries between 80 and 120% of their anticipated value and a response at least five times that of the control samples.

Precision (within-Day, between-Day), Accuracy, and Recovery. *Within-Day Precision.* The precision and accuracy within-day of the method for each matrix was determined by analyzing six control samples fortified at the LLOQ, six control samples fortified at 20 times the LLOQ, and six control samples fortified at 200 times the LLOQ. Good within-day precision was demonstrated with coefficients of variation (CVs) <15% for C8, C9, C10, C11, and C13 at each fortification level.

Accuracy and Recovery. The accuracy of the method was verified with mean value recoveries within 15% from the actual concentrations for C8, C9, C10, C11, and C13 at each fortification level. All recoveries were within 20% of the actual concentrations.

Between-Day Precision. Another set containing six control samples fortified at 20 times the LLOQ was extracted by a second person on a separate day. Comparing results for the 20-times LLOQ fortifications from the first day with those obtained on the second day established the between-day precision with CVs of mean recoveries of <15%.

Absolute Recovery. The percent absolute recovery was based on a standard curve generated using nonextracted standards representing 100% recovery. For each matrix, three control samples fortified at the LLOQ, three control samples fortified at 20 times the LLOQ, and three control samples fortified at 200 times the LLOQ were compared to a set of nonextracted standards to establish absolute recovery.

The mean recoveries were within 20% of the actual concentrations for C8, C9, C10, C11, and C13 at each fortification level and within 15% for the entire set. The average absolute recovery and

CV for the 9H surrogate standard was 188 ± 16%. The high 9H recovery may be due to matrix enhancement, but was considered acceptable because the recovery was consistent and precise (CV is < 20%). All of the 250-ng/L 9H recoveries in the nondiluted extracted samples throughout the study were between 70 and 130%.

Sample and Standard Stability. *Short-Term Room-Temperature Stability of Samples and Extracts.* The short-term stability of the samples and extracts during the extraction and analysis procedure was determined by fortifying six control samples at 50 ng/L and six at 2500 ng/L for all analytes. Three replicates at each level were immediately extracted along with a set of standards and analyzed. Mean recoveries were within 20% of the actual concentrations for C8, C9, C10, C11, and C13 at each fortification level. After analyzing, the initial sample and standard extracts were allowed to remain at room temperature for ~24 h and then reinjected. Mean recoveries were still within 20% of the actual concentrations at each fortification level. The other fortified samples that were not immediately extracted were allowed to remain at room temperature for ~24 h. Following this period, they were extracted and analyzed with a calibration curve from a fresh set of extracted standards. These samples also had mean recoveries within 20% of the actual concentrations for C8, C9, C10, C11, and C13 at each fortification level.

A fresh set of calibration standards was prepared and compared to those that sat at room temperature for ~24 h. The standards also demonstrated stability for ~24 h at room temperature. The average recovery and standard deviation for the standard comparison was 87 ± 4% for C8, 88 ± 6% for C9, 85 ± 6% for C10, 87 ± 5% for C11, 91 ± 7% for C13, and 96 ± 3% for 9H.

Long-Term Refrigerator Stability of Standards and Extracts. A set of calibration standards having concentrations from 0 to 2500 ng/L, three fortified samples at 50 ng/L, and three at 2500 ng/L for all analytes were stored refrigerated between 2 and 6 °C, and they were compared to freshly prepared standards at 1- and 2-week intervals. For the standards, recoveries were within 20% of the actual concentrations for all of standards and the surrogate standard at both 1 and 2 weeks. For the extracts, mean recoveries were within 20% of the actual concentrations for all analytes at both concentrations, and all recoveries were within 30% of actual concentrations. The 1- and 2-week storage stability of the standards and extracts was established.

Long-Term Room-Temperature Stability of Samples. The long-term room temperature stability of the samples was investigated by means of a stability experiment. This consisted of storing samples fortified at 50 and 2500 ng/L for all analytes, in triplicate, for 1, 3, 7, and 14 days. Following the storage period, the samples were analyzed along with a set of freshly prepared calibration standards.

Fortified samples were found to be stable for 14 days for C8, C9, C10, and C11 with mean recoveries within 20% of the actual values when fortified and stored at room temperature. Samples fortified with C13 were found to be stable for only 7 days when stored at room temperature. At 14 days, the mean recoveries and coefficients of variation for C13 were 60 ± 10% at the 50 ng/L fortification level, and 53 ± 9% at the 2500 ng/L fortification level. This compares to 76 ± 13% at the 50 ng/L fortification level and 78 ± 6% at the 2500 ng/L fortification level for C13 at 7 days. The development of inhomogeneity for the longest chain length analyte suggests the occurrence of some process, such as aggregation, that may be related to chain length. The possibility was not examined by further experiment.

Long-Term Refrigerator Stability of Samples. The long-term refrigerator stability of the samples was investigated with an experiment similar to the room-temperature stability experiment. Samples were fortified at 50 and 2500 ng/L in triplicate and stored at 4 ± 2 °C for 1, 3, 7, and 14 days. Following the storage period, the samples were analyzed along with a set of fresh calibration standards.

Fortified samples were found to be stable for 14 days for C8, C9, C10, C11, and C13 when stored refrigerated at 4 ± 2 °C, with mean recoveries within 20% of the actual values. Development of inhomogeneity in C13 samples was apparently retarded or otherwise did not develop, as was the case in the room temperature stability studies.

Long-Term Stability of Stock Solutions. Methanolic stock solutions which had been stored in a refrigerator at 4 ± 2 °C for long periods were used to prepare calibration standards, which were compared to calibration standards from new stock solutions. The stock solutions for C8 and C9 were found to be stable for at least 313 days, the stock solutions for C10 and C11 for at least 314 days, the stock solution for C13 for at least 85 days, and the stock solution for 9H for at least 183 days, with recoveries within 30% of actual. Methanol is apparently a better solvent for the analytes than is water in that the development of inhomogeneity is not observed in methanol.

CONCLUSIONS

A method for the analysis of C8, C9, C10, C11, and C13 in water using external standards and a surrogate standard was successfully validated.

In absolute recovery experiments, the monohydro surrogate standard appeared to exhibit matrix enhancements, but recoveries were consistent and precise in the absolute recovery experiments, and recoveries of all of the diluted extracted samples (250 ng/L) throughout the study were between 70 and 130%. Therefore, the surrogate standard was still useful in monitoring recovery.

A comparison between the stability study of methanolic stock solutions of the analytes and that of fortified aqueous sample solutions suggests that methanolic solutions of the analytes are more stable than aqueous solutions. Chain length of an analyte may play a role in aqueous solution stability, in that the longest chain length, C13, showed instability in the room temperature stability study of fortified samples, whereas the others were more stable. The stability studies recommend that samples be stored refrigerated and analyzed within 2 weeks of sampling for these types of analytes in water.

SUPPORTING INFORMATION AVAILABLE

Experimental data. This material is available free of charge via the Internet at http://pubs.acs.org.

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